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Patent claims

000181 BT / IP

- An isolated polynucleotide from coryneform bacteria, comprising a polynucleotide sequence which codes for the lysR2 gene, chosen from the group consisting of
- a) polynucleotide which is identical to the extent of at least 70% to a polynucleotide which codes for a polypeptide which comprises the amino acid sequence of SEQ ID No. 2,
 - b) polynucleotide which codes for a polypeptide which comprises an amino acid sequence which is identical to the extent of at least to [sic] 70% to the amino acid sequence of SEQ ID No. 2,
 - c) polynucleotide which is complementary to the polynucleotides of a) or b), and
- d) polynucleotide comprising at least 15 successive nucleotides of the polynucleotide sequence of a), b), or c),

the polypeptide preferably having the activity of the transcription regulator LysR2.

- 20 2. A polynucleotide as claimed in claim 1, wherein the polynucleotide is a preferably recombinant DNA which is capable of replication in coryneform bacteria.
- 3. A polynucleotide as claimed in claim 1, wherein the polynucleotide is an RNA.
 - 4. A DNA as claimed in claim 2 which is capable of replication, comprising
 - (i) the nucleotide sequence shown in SEQ ID No. 1, or

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- (ii) at least one sequence which corresponds to sequence (i) within the range of the degeneration of the genetic code, or
- (iii) at least one sequence which hybridizes with the sequences complementary to sequences (i) or (ii), and optionally
 - (iv) (iv)[sic] sense mutations of neutral
 function in (i) which do not modify the
 activity of the protein/polypeptide.
- 10 5. A polynucleotide as claimed in claim 2, comprising the nucleic acid sequence as shown in SEQ ID No. 1.
 - 6. The vector pCR2.1lysR2int, which
 - 6.1 carries an internal fragment of the citA gene 439 bp in size,
- 15 6.2 the restriction map of which is reproduced in figure 1, and
 - 6.3 which is deposited in the E.coli strain TOP10F/pCR2.1lysR2int under no. DSM 13617 at the Deutsche Sammlung für Mikroorganismen und Zellenkulturen [German Collection of Microorganisms and Cell Cultures].
 - 7. A coryneform bacterium in which the lysR2 gene is attenuated, preferably eliminated, in particular by deletion.
- 25 8. A process for the preparation of L-amino acids, in particular L-lysine and L-valine, which comprises carrying out the following steps,

- a) fermentation of the bacteria which produce the desired L-amino acid and in which at least the lysR2 gene is attenuated,
- 5 b) concentration of the desired product in the medium or in the cells of the bacteria and
 - c) isolation of the L-amino acid.
 - A process as claimed in claim 8, wherein
- 10 bacteria in which further genes of the biosynthesis pathway of the desired L-amino acid are additionally enhanced are employed.
 - 10. A process as claimed in claim 8, wherein
- bacteria in which the metabolic pathways which reduce the formation of the desired L-amino acid are at least partly eliminated are employed.
 - 11. A process as claimed in claim 8, wherein
- expression of the polynucleotide(s) which codes (code) for the lysR2 gene is decreased, in particular eliminated.
 - 12. A process as claimed in claim 8, wherein
- 25 the regulatory properties of the polypeptide for which the polynucleotide lysR2 codes are decreased.
 - 13. A process as claimed in claim 8, wherein
- for the preparation of L-amino acids, in particular L
 lysine, bacteria in which at the same time one or more

 of the genes chosen from the group consisting of

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- 13.1 the dapA gene which codes for dihydrodipicolinate synthase,
- 13.2 the eno gene which codes for enolase,
- 13.3 the zwf gene which codes for the zwf gene product,
 - 13.4 the pyc gene which codes for pyruvate carboxylase,
 - 13.5 the lysE gene which codes for lysine export,
 - 13.6 the lysC gene which codes for a feed-back resistant aspartate kinase
 - 13.7 the zwal gene which codes for the Zwal protein is or are enhanced, preferably over-expressed, are fermented.
 - 14. A process as claim in claim 8, wherein at the same time one or more of the genes chosen from the group consisting of:
 - 14.1 the pck gene which codes for phosphoenol pyruvate carboxykinase,
- 20 14.2 the pgi gene which codes for glucose 6-phosphate isomerase,
 - 14.3 the poxB gene which codes for pyruvate oxidase,
 - 14.4 the zwa2 gene which codes for the Zwa2 protein,
- 14.5 the hom gene which codes for homoserine dehydrogenase
 - 14.6 the thrB gene which codes for homoserine kinase, and

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14.7 the panD gene which codes for aspartate decarboxylase

is or are attenuated, in particular eliminated.

- 15. A process as claimed in claim 8,
- 5 wherein

for the preparation of L-amino acids, in particular L-valine, bacteria in which at the same time one or more of the genes chosen from the group consisting of

- 15.1 the ilvBN gene which codes for acetohydroxy-acid synthase,
- 15.2 the ilvD gene which codes for dihydroxy-acid dehydratase,
- 15.3 the mgo gene which codes for malate:quinone oxidoreductase
- is or are enhanced, in particular over-expressed, are fermented.
 - 16. A process as claimed in one or more of the preceding claims,

wherein

- 20 microorganisms of the species Corynebacterium glutamicum or Brevibacterium lactofermentum are employed.
- 17. A process for discovering RNA, cDNA and DNA in order to isolate nucleic acids, or polynucleotides or genes which code for the transcription regulator LysR2 or have a high similarity with the sequence of the lysR2 gene,

which comprises

employing the polynucleotide sequences as claimed in claims 1 to 4 as hybridization probes.

18. A process as claimed in claim 15, wherein arrays, micro arrays or DNA chips are employed.